# 2P4-7

## Discrimination of HeLa Cells Using Ultrasound Vibration

超音波振動による HeLa 細胞の判別

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### 1. Introduction

In vitro analysis of biological samples is a powerful tool to inspect individual cell and DNA and predict reactions in the body. However, biological tests such as blood sampling and gene analysis are based on the average value obtained from enormous amount of samples. In these diagnostic methods, there is a risk in missing diseases in their early stage since these average results including the intact samples dilute the differences among individual cells [1]. In order not to miss, it is necessary to diagnose in units of cells. So techniques to select, manipulate and analyze a micro biological material with high accuracy and selectivity are required in the fields of medical and life sciences. Many researchers have reported manipulation techniques of small objects using small chips with microstructures (Lab-on-a-chip) so far [2,3].

Our group has also been investigating noncontact ultrasonic manipulation techniques using acoustic standing wave. In this report, a method to discriminate HeLa cells using an ultrasound standing wave was proposed. The difference of the characteristics between dead and live HeLa cells in the acoustic field were investigated.

### 2. Methods

**Fig. 1** shows the configuration of an ultrasonic chip for cell manipulation. Two ultrasound PZT transducers (10.0×10.0×1.0 $mm^3$) were attached to both ends of a glass substrate (60.0×10.0×2.0 $mm^3$) with a microchannel (40.0×1.0×1.0 $mm^3$) filled with suspension of HeLa cells (average 15 $\mu$m in diameter) and culture medium (E-MEM). The configuration of the glass substrate, the transducers, and the channel were determined by finite element analysis (FEA, ANSYS 14.5) to obtain larger vibration amplitude at the bottom of the channel. When the flexural vibration is excited along the channel, the acoustic standing-wave field is generated in the channel. The HeLa cells which are much smaller than the wavelength of sound wave can be trapped along the nodal lines of the acoustic standing wave by the acoustic radiation force.

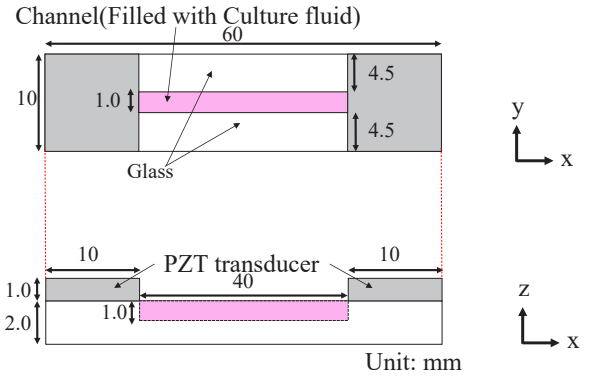


Fig. 1 Configuration of an ultrasound chip for micro-manipulation.

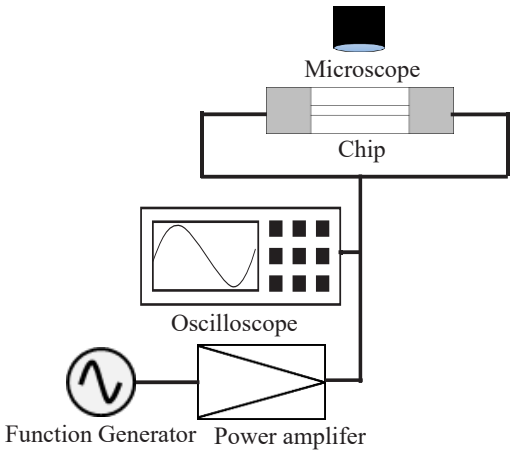


Fig. 2 Experimental setup.

The experimental system is shown in **Fig. 2**. A continuous sinusoidal signal generated by a function generator (NF, WF1946A) was amplified by a power amplifier (E&I, 350L), and the electric signal was input to each transducer. The behavior of HeLa cells in the microchannel was observed using a microscope (OLYMPUS, IX83).

### 3. Results and Discussion

**Fig. 3** shows a photograph of the HeLa cells at the bottom of the channel under ultrasound excitation when an in-phase continuous sinusoidal wave at 510 kHz and 30 $V_{pp}$ was applied to each transducer. The cells were trapped along the nodal lines of the acoustic standing wave in the channel

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with equal intervals of 2 mm which correspond to half the wavelength of the standing wave. **Figs. 4(a)** and **(b)** show the photographs of HeLa cells in the channel before and under ultrasound irradiation, respectively. Before ultrasound irradiation (Fig. 4(a)), the cells were distributed uniformly over the channel. By applying the input voltages (Fig. 4(b)), the cells moved gradually and aggregated to the nodal lines of the standing wave by the acoustic radiation force acting on the cells.

Focusing on the transient motion of the cell by ultrasound, a method to discriminate dead cells from live cells was investigated. **Figs. 5(a)** and **(b)** show a photograph of the dead and live cells, respectively. The dead cells were prepared intentionally in the culture process, and the live and dead cells were mixed in the microchannel. The moving speed of the cells in the acoustic standing wave were measured. **Fig. 6** shows the relationship between the cross-sectional areas of the cells calculated from the captured image when cells are focused under the microscope and the moving speed of the live and dead cells in x direction. The moving speed of the cells in the acoustic standing wave depended on the acoustic field in the channel and varied with time since the acoustic radiation force acting to the cells in the standing-wave field was a function of the spatial gradient of the sound pressure amplitude. If the moving speed was determined as the average speed from the default position (0.37 mm away from the nodal position) to the nodal position of the standing wave, those for the live and dead cells were approximately 6.0 to 8.0 and 0.3 to 1.5 $\mu$m/s, respectively. The live cells were trapped rapidly to the nodal position compared with the dead cells, and significant difference was confirmed between the live and dead cells. Considering the difference in density between the inside and the outside of the dead cells, the acoustic radiation force acting to the dead cells will be smaller than that to the live cells since the cellular inclusions might flow out from the cell due to the damage of the cell membrane and the surrounding medium, the culture medium, might flow into the cell.

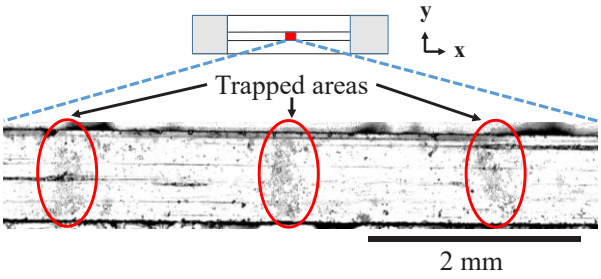


Fig. 3 Photograph of the HeLa cells trapped in the acoustic standing wave in the microchannel at 510 kHz.

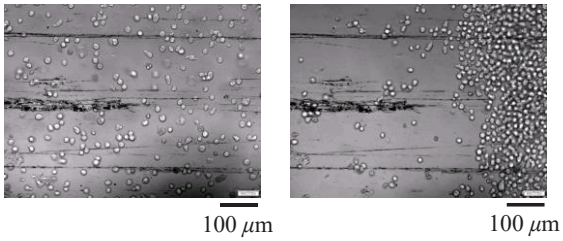


Fig. 4 Photographs of the HeLa cells (a) before and (b) under ultrasound exposure at 510 kHz.

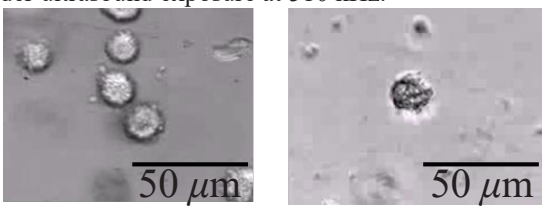


Fig. 5 Photographs of (a) the live and (b) dead HeLa cells.

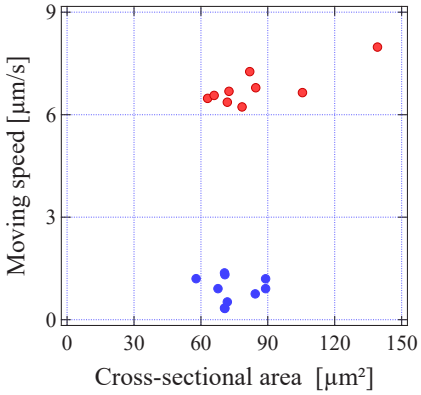


Fig. 6 Relationship between the cross-sectional area and the moving speed of the cells.

## 4. Conclusion

A method to discriminate live HeLa cells from dead cells using an acoustic standing wave in a microchannel was discussed. The results showed a significant difference in the moving speed between the live and dead cells. Next, we intend to investigate a cell separation technique.

**Acknowledgment**

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**References**

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